

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY


(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 13 DEC 2005

WIPO

PC

Applicant's or agent's file reference 6550-086/POA		<b>FOR FURTHER ACTION</b>		See Form PCT/IPEA/416
International application No. PCT/US2004/031417		International filing date (day/month/year) 24.09.2004	Priority date (day/month/year) 24.09.2003	
International Patent Classification (IPC) or national classification and IPC C12N9/88, C12P7/42, C12N15/60				
Applicant BOARD OF TRUSTEES OPERATING MICHIGAN STAT... et al				
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau a total of 5 sheets, as follows:</p> <p><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>				
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input checked="" type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>				
Date of submission of the demand  22.04.2005		Date of completion of this report  12.12.2005		
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized Officer  Bassias, I  Telephone No. +49 89 2399-8106		



**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/US2004/031417

---

**Box No. I Basis of the report**

---

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
  - ☐ publication of the international application (under Rule 12.4)
  - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements\*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

**Description, Pages**

1-37 as originally filed.

**Sequence listings part of the description, Pages**

1-28 as originally filed

**Claims, Numbers**

1-45 received on 22.04.2005 with letter of 22.04.2005

**Drawings, Sheets**

1/5-5/5 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to sequence listing (*specify*):

\* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/US2004/031417

---

**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

---

1. Statement

Novelty (N)	Yes: Claims	1-15,20-22,26-45
	No: Claims	16-19,23-25
Inventive step (IS)	Yes: Claims	3,20-22,26-45
	No: Claims	1,2,4-19,23-25
Industrial applicability (IA)	Yes: Claims	1-45
	No: Claims	-

2. Citations and explanations (Rule 70.7):

**see separate sheet**

---

**Box No. VI Certain documents cited**

---

1. Certain published documents (Rule 70.10)

and /or

2. Non-written disclosures (Rule 70.9)

**see separate sheet**

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/US2004/031417

---

**Supplemental Box relating to Sequence Listing**

---

**Continuation of Box I, item 2:**

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:
  - a. type of material:
    - ☒ a sequence listing
    - ☐ table(s) related to the sequence listing
  - b. format of material:
    - ☒ in written format
    - ☒ in computer readable form
  - c. time of filing/furnishing:
    - ☒ contained in the international application as filed
    - ☒ filed together with the international application in computer readable form
    - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
    - ☐ received by this Authority as an amendment on
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional observations, if necessary:

**Re Item V**

1. The amended claims filed with the letter of 22.04.2005 appear to be allowable under Articles 19(2) and 34(2)(b) PCT.
2. Amended claim 1 was changed by the addition of two different items:
  - a) introduction of the limitation to KDPGal aldolases having a length of about 190 to about 215 residues and
  - b) introduction of the limitation to aldolases having a higher specific activity for DAHP

Item a) is not suitable for overcoming the objections of the previous communication since the aldolases of D1-D4 have a length which falls into the range of about 190 to about 215 residues.

Item b) however, is suitable to overcome the novelty objection raised previously since no such preferred activity is mentioned for the enzymes of D1-D4.

However, claim 1 and all thereto referring claims are still not in accordance with Articles 5, 6 and 33(3) PCT. Although the applicant alleges that KDPGal aldolases having a length of about 190 to about 215 residues belong to a particular group of aldolases with a close functional and structural relationship, one cannot automatically assume that the specific mutations at the positions given lead to a higher specificity for DAHP for every KDPGal aldolase of said particular group.

Said preferred specificity was demonstrated only for the specific aldolases having the sequences as shown in SEQ ID NOs: 2, 4 and 6 but not for any other aldolase.

Hence, a claim referring to any other aldolase is undue broad (Article 6 PCT) and is furthermore not in accordance with Article 5 PCT since other KDPGal aldolases are not sufficiently supported by the description.

---

Furthermore, the technical problem that the mutated KDPGal aldolases have a higher specific activity for DAHP was not convincingly solved for any aldolase belonging to this particular group but only for the specific enzymes as mentioned above.

Consequently, the technical problem was not solved over the whole breadth of the

claim and thus no inventive activity can be acknowledged for such a broad claim (Article 33(3) PCT).

3. The enzymes of D1-D4 comprise at positions 10 and 28 the claimed residues Val (10) and/or Leu (28). Since the claims define merely what amino acid residue should be found at the specific positions, it is irrelevant whether this residue was naturally (wild type) there or was introduced by mutations.
4. Claims 16-19 and 23-25 do not fulfil the requirements of Article 33(2) PCT since the enzymatic pathway is not restricted to a mutated KDPGal aldolase. In this manner such a pathway is not distinguishable from the naturally occurring pathway existing in cells which comprise a wild type KDPGal aldolase and produce shikimate. The introduction of the expression "isolated or recombinant KDPGal aldolase" is not suitable for overcoming said objection. An isolated or recombinant KDPGal aldolase is not necessarily distinguishable from the wild type KDPGal aldolase. The wild type KDPGal aldolase can be as well isolated and the wild type gene cloned in a heterologous expression host results in a recombinant KDPGal aldolase not being different from the wild type enzyme.
5. The expression "about" used in several claims (e.g. claims 1, 7, 8, etc.) is not clear in the sense of Article 6 PCT.

**CLAIMS**

What is claimed is:

1. A recombinant polypeptide that is or contains a KDPGal aldolase of about 190 to about 215 residues in length having at least one of the mutations: X10V, X28L or X28M, X42T, X85A, X154F, or X196I, said KDPGal aldolase having a higher specific activity for 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) formation than the enzyme without said at least one mutation.
2. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has at least one of the mutations: I10V, V28L or V28M, S42T, V85A, V154F, or F196I.
3. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6, and said at least one mutation is a mutation thereto.
4. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has no mutation that is X70L.
5. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has an amino acid sequence at least 50% homologous to that of any of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6, and said at least one mutation is a mutation thereto.
6. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has an amino acid sequence of 190 to 215 residues in length.
7. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has an amino acid sequence about 200 to about 210 residues in length.
8. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has an amino acid sequence about 205 residues in length.
9. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has the amino acid sequence of a native bacterial KDPGal aldolase that has been mutated to contain said at least one mutation.
10. The recombinant polypeptide of Claim 9, wherein said native bacterial KDPGal aldolase is native to a member of the proteobacteria.
11. The recombinant polypeptide of Claim 10, wherein said native bacterial KDPGal aldolase is native to a member of any one of the genera

**BEST AVAILABLE COPY**

*Agrobacterium, Bradyrhizobium, Brucella, Caulobacter, Escherichia, Klebsiella, Ralstonia, Salmonella, and Sinorhizobium.*

12. Nucleic acid encoding a recombinant polypeptide according to any one of Claims 1-11.

5 13. The nucleic acid according to Claim 12, wherein the coding sequence thereof that encodes the KDPGal aldolase of the polypeptide has a nucleotide sequence more than 80% homologous to that of any of SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5.

10 14. The nucleic acid according to Claim 12, wherein said nucleic acid is at least one nucleic acid vector.

15 15. The nucleic acid according to Claim 14, wherein said vector is at least one plasmid.

16. An enzymatic pathway capable of converting pyruvate and D-erythrose 4-phosphate (E4P) into 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP), said pathway including at least one isolated or recombinant KDPGal aldolase.

17. The enzymatic pathway of Claim 16, further comprising at least one DHQ synthase, said pathway being capable of synthesizing 3-dehydroquinate (DHQ) from DAHP.

20 18. The enzymatic pathway of Claim 17, further comprising at least one DHQ dehydratase, said pathway being capable of synthesizing 3-dehydroshikimate (DHS) from DHQ.

25 19. The enzymatic pathway of Claim 18, further comprising at least one shikimate dehydrogenase, said pathway being capable of synthesizing shikimate from DHS.

20. A method for the production of shikimate or a shikimate intermediate comprising (1) providing a recombinant cell containing nucleic acid encoding at least one KDPGal aldolase and at least one DHQ synthase, from which nucleic acid said cell can express those enzymes, and (2) growing said cell in a medium under conditions in which it expresses them; and (3) optionally, recovering at least one of DAHP, DHQ, DHS, or a further derivative thereof, from said medium or from said cell.

21. The method of Claim 20, wherein the shikimate intermediate is at least one of DAHP, DHQ, or DHS.

BEST AVAILABLE COPY



22. The method of Claim 20, wherein said recombinant cell, when grown under said conditions, expresses at least one recombinant transketolase or at least one recombinant transaldolase.

23. A method for converting pyruvate and E4P to DAHP, comprising  
5 contacting an isolated or recombinant KDPGal aldolase with a solution containing pyruvate and E4P.

24. The method of Claim 23, wherein said method further includes contacting said DAHP with a DHQ synthase, thereby forming DHQ.

25. The method of Claim 24, wherein said method further includes  
10 contacting said DHQ with a DHQ dehydratase, thereby forming 3-dehydroshikimate.

26. The method of to any one of Claims 23-25, wherein said method is performed within a recombinant cell.

27. The method of Claim 26, wherein said host cell was produced  
15 by transforming the cell with nucleic acid encoding at least one of a KDPGal aldolase or a DHQ synthase.

28. The method of Claim 26, wherein said recombinant cell contains at least one recombinant transketolase or at least one recombinant transaldolase.

29. Use of a recombinant KDPGal aldolase to produce DAHP from  
20 pyruvate and E4P.

30. The use according to Claim 19, wherein said use further includes use of a recombinant DHQ synthase to produce DHQ from said DAHP.

25 31. A process for preparing a recombinant cell capable of expressing a KDPGal aldolase, and of converting pyruvate and E4P to DAHP by action thereof, comprising:

A) providing a host cell capable of synthesizing pyruvate and E4P,

B) providing a vector containing a polynucleotide from which said host cell can  
30 express a KDPGal aldolase, and

C) transforming said cell with said vector to produce a transformed cell, and, optionally, thereafter expressing said KDPGal aldolase, whereupon said transformed cell converts pyruvate and E4P to DAHP.

BEST AVAILABLE COPY

32. The process according to Claim 31, wherein said KDPGal aldolase has an amino acid sequence at least 50% homologous to that of any one of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6.

5 33. The process according to Claim 32, wherein said KDPGal aldolase has at least one of the mutations: X10V, X28L or X28M, X42T, X85A, X154F, or X196I.

34. A recombinant cell prepared by the process according to any one of Claims 31-33.

35. The cell according to Claim 34, wherein said cell is a walled cell.

10 36. The cell according to Claim 35, wherein said cell is a bacterial cell.

37. The cell according to Claim 34, wherein said cell is an *aroFGH* cell.

15 38. A process for preparing at least one of DAHP or a derivative thereof, said process including the steps of:

1) providing

(A) a supply of E4P and pyruvate,

(B) a KDPGal aldolase, and optionally a DHQ synthase,

(C) an aqueous medium,

20 2) contacting in said medium, said KDPGal aldolase with said E4P and said pyruvate under conditions in which said KDPGal aldolase can catalyze the formation of DAHP from the E4P and pyruvate, and optionally contacting said DAHP with said DHQ synthase under conditions in which said DHQ synthase can catalyze the formation of 3-dehydroquinone from the DAHP;

25 3) optionally recovering at least one of DAHP, DHQ, DHS, or a further derivative thereof, from said medium.

39. A kit containing a KDPGal aldolase preparation, with instructions for the use thereof to convert pyruvate and E4P to DAHP, and optionally with instructions for the conversion of said DAHP to at least one derivative thereof.

30 40. A kit containing a cell capable of expressing a KDPGal aldolase, with instructions for the use thereof to convert pyruvate and E4P to DAHP, and optionally with instructions for the conversion of said DAHP to at least one derivative thereof.

BEST AVAILABLE COPY

41. The kit of Claim 40, wherein said cell is also capable of expressing at least one DHQ synthase.

42. The kit of Claim 41, wherein said cell is also capable of expressing at least one DHQ dehydratase.

5 43. A kit containing nucleic acid from which a cell can express at least one KDPGal aldolase, with instructions for the use thereof to transform a cell to produce a transformed cell that is capable of onverting pyruvate and E4P to DAHP, and optionally to at least one derivative thereof.

10 44. The kit of Claim 43, wherein said kit contains nucleic acid from which a cell can express at least one DHQ synthase.

45. The kit of Claim 43, wherein said derivative of DAHP is at least one of DHQ, DHS, or a downstream derivative of DHS.